The effect of acetazolamide on the kinetics of four newer β-lactams in the aqueous humor

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Objective To evaluate whether the effect of acetazolamide on piperacillin's aqueous humor concentrations observed in animals exists also in humans for ceftazidime, cefotaxime, ceftriaxone and aztreonam.

Methods One hundred and eighty-eight patients undergoing eye cataract surgery were randomly allocated to receive intravenous ceftazidime, cefotaxime, aztreonam or ceftriaxone with (subgroup A) or without (subgroup B) concomitant oral administration of acetazolamide. Antibiotic concentrations in serum and the aqueous humor, simultaneously sampled during the operation, were measured using an agar well diffusion technique, and the ratios of the concentrations of aqueous humor to serum were calculated and compared. Statistical analysis was performed by using the paired *t*-test.

Results Mean aqueous humor ceftazidime concentrations at 2, 4 and 6 h were 24.65, 16.4 and 8.6 mg/L (subgroup A), and 4.26, 8.66 and 5.61 mg/L (subgroup B). Corresponding concentrations of cefotaxime were 1.75, 1.0 and 0.77 mg/L (subgroup A), and 1.11, 0.81 and 0.58 mg/L (subgroup B), and of aztreonam 6.9, 5.84 and 3.61 mg/L (subgroup A), and 3.38, 2.57 and 1.48 mg/L (subgroup B). Ceftriaxone concentrations at 2, 4, 6 and 12 h were 1.78, 1.49, 1.57 and 1.41 mg/L (subgroup A), and 1.35, 0.95, 1.08 and 0.85 mg/L (subgroup B). The differences in aqueous humor concentrations when acetazolamide was administered were statistically significant ($P < 0.05$), with the exception of ceftazidime 6 h, cefotaxime 6 h and ceftriaxone 2 h.

Conclusions Although acetazolamide resulted in statistically significant increases in the aqueous humor concentrations of all the antibiotics tested, this effect was most marked for ceftazidime.

Keywords Endophthalmitis, acetazolamide, cephalosporins, ceftazidime, cefotaxime, ceftriaxone, aztreonam, pharmacokinetics

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INTRODUCTION

Bacterial endophthalmitis is a suppurative inflammation of the intraocular contents in which not all the layers of the globe are affected and in which the eye does not rupture. Endophthalmitis may be either exogenous, following penetrating wounds, either accidental or surgical, or the insertion of a foreign body, or endogenous, resulting either from a distant focus of infection through hematogenous spread or from a nearby focus by direct spread [1]. It is a severe infection, as the probability of resulting blindness is high. Coagulase-negative staphylococci are now the most commonly involved pathogens, followed by *Staphylococcus aureus, Pseudomonas aeruginosa,* other Gram-negative rods and streptococci. Moreover, bacteria previously not considered as pathogens, such as *Bacillus cereus* (mostly associated with trauma), *Propiobacterium acnes*, and fungi, are now being isolated with increasing frequency.

Despite the development of new and potent antibiotics in recent years, it is now clear that fully efficient therapy for such infections does not exist because of the poor penetration of systemic antibiotics into the intraocular compartments, due to the existing barriers [2,3]. The situation is further complicated by the fact that resistance has already emerged in the commonly implicated pathogens, even to the newer agents. While some newer and promising antibiotics active against Gram-positive cocci, such as linezolid and synercid, have recently been introduced, it still holds true that such drugs active against Gram-negative rods will not be available in the near future [4].

One solution could be to maximize the benefit from the existing antimicrobials by improving their eye kinetics. Previous

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Table 1 Demographic characteristics of our patients

	Group I (Ceftazidime)		Group II (Cefotaxime)		Group III (Ceftriaxone)		Aztreonam	
	A	B	A	B	A	B	A	B
No. of patients	31	15	22	22	25	24	26	23
Male	21	10	14	11	12	13	11	15
Female	10	5	8	11	13	11	15	8
Mean age (years)	72.3	70.3	71.8	73.4	71.8	75.1	74.6	71.3

A, with acetazolamide; B, without acetazolamide.

work in animals [5] has shown that acetazolamide administration is effective in increasing the aqueous humor concentrations of piperacillin but not of tobramycin. In the present study, we tried to investigate whether the above finding could apply also to the human eye by studying acetazolamide effects upon the aqueous humor kinetics of four newer β-lactams, namely ceftazidime, cefotaxime, ceftriaxone and aztreonam, active against multiresistant Gram-negative bacteria of nosocomial origin.

PATIENTS AND METHODS

One hundred and eighty-eight patients, 107 males and 81 females, undergoing eye cataract extraction were included in this open randomized study, after giving oral informed consent. Their age range was 47–92 years, mean 73.2 years. The cataracts were classified as senile in 177, presenile in nine, and pathologic in two. Excluded from the study were patients currently being, or having been for the preceding 2 weeks, either locally (ointments or eye solutions) or systemically treated with antibiotics for any reason, patients with renal failure (serum creatinine >1.5 mg/dL), liver insufficiency (serum AST or ALT >2 times upper normal limits), or known allergy to β-lactams, patients in poor general condition, and those not consenting. Patients were randomized, according to a predetermined order generated by using allocation tables, into four groups (I–IV), according to the antibiotic given. Group I consisted of patients receiving ceftazidime, group II of those receiving cefotaxime, group III of those receiving ceftriaxone, and group IV of those receiving aztreonam. Each group was further divided into two subgroups according to whether acetazolamide was concurrently given (A) or not given (B).

The investigation was approved by the Hospital Ethics Committee, and the research was carried out in accordance with the Helsinki Declaration.

All antibiotics were given as a 30-min intravenous infusion of two 2-g doses diluted into 100 mL of normal saline, 6 h (ceftazidime), 8 h (cefotaxime, aztreonam) or 12 h (ceftriaxone) apart, the last being administered at 2 h, 4 h and 6 h (ceftriaxone also at 12 h, because of its greater half-life) before the operation. Acetazolamide (subgroup A patients only) was administered orally as three 250-mg doses at 6-h intervals, the last dose being given 3 h before the scheduled time of operation, which is the anticipated time of its peak concentration [6].

The demographic characteristics of patients allocated to each group are shown in Table 1, while the numbers of patients sampled at each time interval are shown in Table 2. Therefore, randomization resulted in group construction without any significant difference regarding patients' demographics.

Sampling of the aqueous humor was done through puncture at the surgical corneoscleral limbus, using a fine needle and syringe at the beginning of the operation and after local anesthesia but before the incision was performed. Operative field preparation was done using povidone iodine solution, and the eye was washed with Balanced Salt Solution (BSS—Alcon Couvrier SA, Purs, Belgium). No antibiotic-containing pre-

Table 2 Number of patients sampled at various time intervals after the last antibiotic administration

	Group I (Ceftazidime)		Group II (Cefotaxime)		Group III (Ceftriaxone)		Group IV (Aztreonam)	
Time (h)	A	B	A	B	A	B	A	B
2	11	5	6	6	7	6	9	2
4	10	5	9	10	7	6	9	4
6	10	5	7	6	6	6	8	6
12					5	6		
Total	31	15	22	22	25	26	26	23

Table 3 Results of the effects of acetazolamide on aqueous humor kinetics of ceftazidime, cefotaxime, ceftriaxone and aztreonam in humans with non-inflamed eye

	Group I: Ceftazidime (mg/L)		Group II: Cefotaxime (mg/L)		Group III: Ceftriaxone (mg/L)		Group IV: Aztreonam (mg/L)	
Time (h)	A	B	A	B	A	B	A	B
2								
H	22.4 (± 16.6)	4.26 (± 1.75)	1.75 (± 0.36)	1.11 (± 0.28)	1.76 (± 0.39)	1.35 (± 0.11)	6.9 (± 1.26)	3.38 (± 0.83)
S	73.36 (± 47.5)	66.4 (± 24.8)	15.6 (± 1.57)	11.35 (± 2.84)	61.0 (± 11.0)	52.67 (± 6.65)	55.11 (± 6.92)	49.37 (± 6.27)
R	0.30	0.06	0.11	0.10	0.03	0.025	0.12	0.06
H	16.4 (± 9.2)	8.66 (± 5.49)	1.0 (± 0.22)	0.81 (± 0.23)	1.49 (± 0.3)	0.95 (± 0.26)	5.64 (± 1.16)	2.57 (± 0.63)
4								
S	38.5 (± 13.3)	51.8 (± 11.9)	4.8 (± 0.77)	3.59 (± 0.72)	45.14 (± 5.12)	42.33 (± 4.1)	33.66 (± 5.51)	26.12 (± 5.24)
R	0.43	0.17	0.22	0.225	0.033	0.022	0.17	0.1
H	8.6 (± 5.5)	5.61 (± 7.5)	0.77 (± 0.15)	0.58 (± 0.24)	1.57 (± 0.34)	1.08 (± 0.23)	3.61 (± 0.49)	1.48 (± 0.3)
6								
S	34.4 (± 14.9)	30.6 (± 30.5)	1.73 (± 0.39)	1.88 (± 0.49)	40.16 (± 2.61)	48.0 (± 3.69)	27.31 (± 2.93)	21.0 (± 3.4)
R	0.25	0.18	0.44	0.35	0.039	0.022	0.13	0.07
H	ND	ND	ND	ND	1.41 (± 0.42)	0.85 (± 0.16)	ND	ND
12								
S	ND	ND	ND	ND	28.8 (± 5.72)	23.6 (± 2.0)	ND	ND
R	ND	ND	ND	ND	0.04	0.036	ND	ND

H, mean aqueous humor concentration (± SD); S, mean serum concentration (± SD); R, corresponding ratios of H to S; A, with acetazolamide; B,without acetazolamide; ND, not done.

paration was used on either eye before puncture. Local anesthesia was obtained by truncal ipsilateral facial nerve blocking and retrobulbar injection of 2% xylocaine and 0.5% marcaine solutions. Puncture was performed at the superior part of the sclerocorneal junction with a fine needle and syringe, and the aqueous humor sample thus obtained was transferred into a sterile container. A 5-mL venous blood sample was simultaneously withdrawn. Serum was aseptically separated, and both samples were kept at $-70°C$ until they were assayed. Antibiotic assays were performed using an agar well diffusion technique with Sensitivity Test Agar no. 2 (Gibco BRL, Paisley, UK) and *Escherichia coli* 14 (ICB 4004) as indicator organism [7].

Statistical analysis of the results was done using the paired *t*-test.

RESULTS

Mean concentrations and standard deviations (SDs) in aqueous humor (H) and serum (S) and the corresponding ratios (R) of aqueous humor to serum concentrations for each group of patients are shown in Table 3.

All aqueous humor concentrations achieved after acetazolamide administration were increased. Statistical significance was obtained for ceftazidime at 2 h ($P < 0.01$) and 4 h ($P < 0.05$) but not at 6 h, for cefotaxime at 2 h ($P < 0.01$) and 4 h ($P < 0.05$) but not at 6 h, for ceftriaxone at 4 h ($P < 0.01$), 6 h ($P < 0.05$) and 12 h ($P < 0.05$), and for aztreonam at all the intervals tested ($P < 0.001$).

DISCUSSION

Endophthalmitis remains a serious infection that threatens the patients' vision and may lead to enucleation of the bulb. Although new and potent antimicrobial agents with improved pharmacokinetic properties have been developed during the last few years, bacteria have already developed resistance mechanisms to them, and no alternatives, especially for Gram-negative rods, are likely to be available in the near future. Such infections are also more problematic to treat, since several of them are now recognized to be caused by bacteria previously considered to be non-pathogenic, such as *Bacillus cereus and Propiobacterium acnes*, some of which are also multiply resistant. The blood–eye barriers further compromise the efficacy of systemic antibiotics, necessitating their local administration in the form of eye drops, or as subconjunctival or parabulbar injections. The latter practice, although remaining the mainstay of the current therapeutic approach, has limited usefulness, since pain and local irritation, as well as local toxicity problems, usually make impossible the repetition of this form of administration as often as needed. Direct intravitreous injection of antibiotics is currently being used together with locally applied antibiotics for treating postoperative endophthalmitis in order to achieve higher antibiotic concentrations within the inflamed eye. Vitrectomy, according to the results of a recently published large trial, must be reserved for patients not responding to conservative therapy within 36–60 h or for those presenting with only light perception [8]. From the same study, it was also concluded that systemically administered ceftazidime–amikacin is of limited benefit, and the decision regarding its administration must be based on clinical judgment. One solution would be to find ways of improving the use of the currently existing antibiotics in order to maximize the expected benefit of their systemic use in the therapy of serious intraocular infections.

Previous work in animals [5] has shown that co-administration of acetazolamide could result in a clinically significant increase of the aqueous humor concentration of piperacillin but not of tobramycin. Change of the intraocular pH and/or dehydration of the bulb caused by acetazolamide, in addition to its physicochemical properties, and especially the electrical charges of the individual antibiotics, are offered as possible explanations of these effects. In the present study, the possibility of the extension of this effect to the human eye was explored by studying the effects of acetazolamide upon the eye pharmacokinetics of four newer β-lactams, namely ceftazidime, cefotaxime, ceftriaxone and aztreonam, which were selected to be studied because of their broad spectrum of activity, which permits therapy of nosocomial infections.

Human steady-state pharmacokinetic studies of ceftazidime in 11 patients after three 1-g intramuscular doses disclosed mean aqueous humor levels of 3.3–1.2 mg/L 2–6 h later [9], while similar levels have been reported (3.39–1.94 mg/L, 0.5–6 h) after a single 2-g intravenous dose [10]. In the present study, levels substantially higher, most evident at 4 h and especially 6 h, were obtained for our patients in the non-acetazolamide (group I_B, ceftazidime) group, and this has to be attributed to the route and the larger dose administered. Acetazolamide resulted in statistically significantly higher levels at 2 and 4 h that exceeded even ceftazidime's susceptibility cut-off point for *P. aeruginosa* (16 mg/L). Since the ratio of aqueous humor to serum concentrations increased 2–6-fold, one has to consider this as a specific effect of acetazolamide upon the eye and not just a consequence of higher serum levels.

Studies of cefotaxime pharmacokinetics in humans showed that intramuscular injection of 1 g (in 25 people) resulted in undetectable aqueous humor levels, while subconjunctival injection of 100 mg in the same patients resulted in detectable levels only in those who were lenseless or had undergone vitrectomy [11]. Intravenous administration of three 1- or 2-g doses in patients undergoing eye cataract surgery resulted in mean maximum aqueous humor concentrations of 1.85 mg/L and 3.8–3.95 mg/L [9,12], respectively, obtained about 2 h later, and administration of 2 g, intravenously, 2 h before vitrectomy in mean intravitreal concentrations of 0.95 mg/L

[13]. The observed difference must reflect the higher cefotaxime serum levels obtained after intravenous administration. These results are in accordance with that for our group II_B (cefotaxime) patients. Although acetazolamide resulted in statistically significantly higher aqueous humor concentrations of cefotaxime at 2 and 4 h, from the clinical point of view these had to be considered negligible, the observed rise being attributed to the higher resulting corresponding serum levels, since the ratio of aqueous humor to serum concentrations remained mostly unchanged.

Intravenous administration of ceftriaxone in humans did not produce detectable levels [14] after three 1-g doses, although the authors could not exclude the possibility that this could be ascribed to the assay they applied. In the present study, low levels, but still useful for treating infections due to highly susceptible pathogens, e.g. enterobacteriaceae, were found even in patients not receiving acetazolamide. The latter had a statistically significant effect upon ceftriaxone aqueous humor concentrations. From the clinical point of view, this could be considered indicative of some benefit, since it resulted in prolongation of ceftriaxone persistence in this compartment, so that concentrations at 4, 6 and 12 h were kept close to those at 2 h. The mechanism of this effect remained obscure, since inconsistent changes in the aqueous humor to serum ratios were found.

Studies of aztreonam kinetics in human eye [15] revealed mean aqueous humor levels, 1–4 h after a 2-g intravenous infusion, of 1.22–2.22 mg/L. These levels far exceeded the MICs for most Enterobacteriaceae, with the exception of *Enterobacter* spp., but not that of *Pseudomonas aeruginosa*. In the present study, acetazolamide resulted in a more than doubling of aztreonam aqueous humor concentrations that was statistically significant at all the intervals tested and could be of clinical relevance.

Although the anterior chamber is not representative of the other eye compartments, it can be considered the most crucial, since at least some of the intraocular infections that start there, such as those resulting from corneal trauma or those associated with eye cataract surgery, can, with prompt treatment, be confined there. From the clinical point of view, our findings suggest that the increase in aqueous humor concentrations obtained by acetazolamide co-administration can be considered relevant mostly for ceftazidime and perhaps for aztreonam and ceftriaxone, the effect on the latter being more prominent for the delayed concentrations, and entirely irrelevant for cefotaxime.

Taking into account the fact that in order for there to be a beneficial effect, an antibiotic's level at the site of infection has to exceed the antibiotic's MIC for the pathogen isolated, the present study identified a clinically significant benefit from acetazolamide co-administration only for ceftazidime and not with any of the remaining three β-lactams. The different behavior of ceftazidime is difficult to explain. A possible explanation might be that the position 3 substitute of ceftazidime, being highly polar, is greatly influenced, according to the Henderson–Hasselbach equation, by the aqueous humor pH shift produced by acetazolamide, resulting in a delay in its aqueous humor clearance, in a similar way to that suggested for piperacillin [5]. However, it must be taken into account that the present study was performed in non-inflamed eyes, and the above results may not apply to already inflamed eyes, where eye barriers are destroyed.

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